

Declaration by A. Bubert

In re application of Schubert et al.

Serial No.: 09/372,036

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Schubert et al.

Serial No.: 09/372,036

Filed June, 06, 1993

For: PROCESS AND AGENTS FOR DETECTING LISTERIAS

Group Art Unit: 1645

Examiner: Baskar, P.

DECLARATION

Honorable Commissioner of
Patents and Trademarks
Washington, D.C., 200231

MADAM/SIR:

The Declarant, Andreas Bubert, being duly warned, declares and says:

THAT he is a German citizen, residing at 64401 Gross-Bieberau, Germany;

THAT he is a biologist having studied at the University of Würzburg, Germany, from 1981 to 1988;

THAT he graduated from the University of Würzburg in 1988,

THAT he obtained the Dr. rer. nat. degree in the field of microbiology and biochemistry from the University of Würzburg in 1993;

THAT he worked as a postdoctoral research associate at the University of Würzburg from 1993 to 1998;



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THAT, in 1998 he joined the Research and Development Department of the Scientific Laboratory Products Division of MERCK KGaA, Darmstadt, Germany;

THAT, since 1992 he has been working in the fields of immunology and immunologic testing, including *Listeria* and *Salmonella*;

THAT he is author or co-author of numerous articles in the field of immunology and immunologic testing;

THAT he is inventor or co-inventor of numerous inventions in the field of immunology and immunologic testing;

THAT he is familiar with issues related to immunology and immunologic testing, especially with the subject invention disclosed and claimed in U.S. Patent Application Ser. No. 09/372,036, filed June 06, 1993, by P. Schubert et al (hereinafter referred to as Application), of which he is co-inventor;

THAT the procedure according to Kohler does not result in one single kind of antibody, but in a mixture containing a plurality of different antibodies with different specificities. The antiserum raised against purified p60 according to Kohler may also contain antibodies that bind an epitope from the peptides according to SEQ. ID. NO: 17, 20, 26, 29, 30 or 31.

THAT even though the antibodies specific for the claimed epitopes might be present in the mixture produced according to Kohler, it is not possible to isolate them without having the information presented in the present invention. If one does not know to which epitope an antibody within a mixture of different antibodies binds, one is not able to reproducibly identify or isolate it.

THAT the teaching of Lerner et al. that peptides derived from the sequence of the whole protein can be used to raise antibodies is not generally applicable. The view of Lerner is also disapproved in literature (see declaration of S. Neumann, April, 1994).

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THAT only very few peptides out of numerous peptides whose sequence is specific for L. monocytogenes are suitable for the preparation of specific antibodies.

Certainly a person skilled in the art would not have had a reasonable expectation of success of generating antibodies having the claimed specificities.

THAT no hint is given in any document of prior art that the peptides claimed in the present invention are suitable for the generation of antibodies specific for L. monocytogenes.

THAT the data shown in Appendix 1 demonstrate that out of a group of several peptides which could be selected for the generation of antibodies only a few peptides are successful for generating specific antibodies.

THAT the undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the APPLICATION or any patent issuing thereon.

Done, this November 08, 2001, at Darmstadt, Germany



Dr. Andreas Bubert

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APPENDIX 1

1. Production of Antibodies

The peptides A-H have been prepared essentially as described in Example 9 of the present application.

The peptides were conjugated with glucose dehydrogenase from *Bacillus megaterium*. The conjugates were used for immunisations.

Antisera against the conjugates containing the peptides were produced essentially as described in Example 11 of the present application.

Peptide	Name in sequence listing	Resemblance to SEQ ID Nr.	Peptide Sequence
A	Pep D/A4B6	[20]	C-QQQTAPKAPTE
B	A2	[17]	C-STPVAPTQEVKK
C	Mono1-Pep		C-QVNNEVAAAEKTEK
D	Mono3-Pep		C-KLAIKQTANTATPK
E	52-8		C-ETKETPVVDQN
F	52-9		C-PKVAETKETP
G			C-QQAAPAAETK
H			C-TNTNTNNTNTN

The position of the sequence of the peptides A-H within the p60 sequence is shown in the attached sequence listing.

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2. Analysis of the Antibodies

The antisera raised against the peptides were analysed both with the peptide used for immunisation (positive control) and with proteins from culture supernatants of *L. monocytogenes* (containing the p60 protein).

Peptide	Immunisation In:	Recognition: Peptide (Pos. Ctrl)	Recognition: p60 by Immunoblot	Specific for L. mono. p60
A	RABBIT	YES	YES	YES
B	RABBIT	YES	YES	YES
C	RABBIT	YES	NO	NO
D	RABBIT	YES	NO	NO
E	RABBIT	YES	YES	NO
F	RABBIT	YES	YES	NO
G	RABBIT	YES	YES	NO
H	RABBIT	YES	YES	NO

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1437 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Listeria monocytogenes*

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION:1..1437

(xi) SEQUENCE DESCRIPTION:

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                                GT CGA
                                11a; [9]

ATG AAT ATG AAA AAA GCA ACT ATC GCG GCT ACA GCT GGG ATT GCG GTA      48
CTG AAT ATG AAA AAA GCA AC      11a; [9]
  AAT ATG AAA AAA GC      1a; [1]
  X1 AAT ATG AAA AAA GC X2      Va
                                GCT ACA GCT GGG ATT GCG GT      11c; [11]
Met Asn Met Lys Lys Ala Thr Ile Ala Ala Thr Ala Gly Ile Ala Val
  1              5              10              15

ACA GCA TTT GCT GCT CCA ACA ATC GCA TCC GCA AGC ACT GTA GTA GTC      96
Thr Ala Phe Ala Ala Pro Thr Ile Ala Ser Ala Ser Thr Val Val Val
          20              25              30

GAA GCT GGT GAT ACT CTT TGG GGT ATC GCA CAA AGT AAA GGG ACT ACT      144
Glu Ala Gly Asp Thr Leu Trp Gly Ile Ala Gln Ser Lys Gly Thr Thr
          35              40              45

GTT GAC GCA ATT AAA AAA GCA AAC AAT TTA ACA ACA GAT AAA ATC GTA      192
Val Asp Ala Ile Lys Lys Ala Asn Asn Leu Thr Thr Asp Lys Ile Val
          50              55              60

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Listeria iap-/p60

CCA GGT CAA AAA TTA CAA GTA AAT AAT GAG GTT GCT GCT GCT GAA AAA	240
TA ACT GAG GTA GCG AGC GAA	IIf; [14]
CT GAG GTA GCG AGC	If; [6]
X1 CT GAG GTA GCG AGC X2	Vf
Pro Gly Gln Lys Leu Gln Val Asn Asn Glu Val Ala Ala Ala Glu Lys	
65 70 75 80	
Gln Val Asn Asn Glu Val Ala Ala Ala Glu Lys	(3); [39]
Gln Val Asn Asn Glu Val Ala Ala Ala Glu Lys	(2g); [32]
Val Asn Asn Glu Val Ala Ala Ala Glu Lys	IIIe; [21]
X3 Val Asn Asn Glu Val Ala Ala Ala Glu Lys	Ive
Gln Val Asn Asn Glu Val Ala Ala Ala Glu Lys	Mono1-Pep

ACA GAG AAA TCT GTT AGC GCA ACT TGG TTA AAC GTC CGT ACT GGC GCT	288
Thr Glu Lys Ser Val Ser Ala Thr Trp Leu Asn Val Arg Thr Gly Ala	
85 90 95	
Thr Glu Lys Ser Val Ser Ala Thr Trp Leu Asn Val Arg Thr Gly Ala	(3); [39]
Thr Glu Lys	(2g); [32]
Thr Glu	IIIe; [21]
Thr Glu X4	Ive
Thr Glu Lys	Mono1-Pep

GGT GTT GAT AAC AGT ATT ATT ACG TCC ATC AAA GGT GGA ACA AAA GTA	336
Gly Val Asp Asn Ser Ile Ile Thr Ser Ile Lys Gly Gly Thr Lys Val	
100 105 110	
Gly Val Asp Asn Ser Ile Ile Thr Ser Ile Lys Gly Gly Thr Lys Val	3); [39]

ACT GTT GAA ACA ACC GAA TCT AAC GGC TGG CAC AAA ATT ACT TAC AAC	384
Thr Val Glu Thr Thr Glu Ser Asn Gly Trp His Lys Ile Thr Tyr Asn	
115 120 125	
Thr Val Glu Thr Thr Glu Ser Asn Gly Trp His Lys Ile Thr Tyr Asn	3); [39]

GAT GGA AAA ACT GGT TTC GTT AAC GGT AAA TAC TTA ACT GAC AAA GCA	432
Asp Gly Lys Thr Gly Phe Val Asn Gly Lys Tyr Leu Thr Asp Lys Ala	
130 135 140	
Asp Gly Lys Thr Gly Phe Val Asn Gly Lys Tyr Leu Thr Asp Lys Ala	(3); [39]

GTA AGC ACT CCA GTT GCA CCA ACA CAA GAA GTG AAA AAA GAA ACT ACT	480
ACT AGC ACT CCA GTT GGT TAA AC	IIg; [15]
AGC ACT CCA GTT GGT TA	Ig; [7]
X1 AGC ACT CCA GTT GGT TA X2	Vg
Val Ser Thr Pro Val Ala Pro Thr Gln Glu Val Lys Lys Glu Thr Thr	
145 150 155 160	
Val Ser Thr Pro Val Ala Pro Thr Gln Glu Val Lys Lys Glu Thr Thr	(3); [39]
Val Ser Thr Pro Val Ala Pro Thr Gln	(2a); [26]
Val Ser Thr Pro Val Ala Pro Thr Gln Glu Val Lys Lys	(2e); [30]
Pro Val Ala Pro Thr Gln Glu Val Lys Lys	(2f); [31]
Pro Val Ala Pro Thr Gln	IIIa; [17]
X3 Pro Val Ala Pro Thr Gln X4	Iva
Ser Thr Pro Val Ala Pro Thr Gln Glu Val Lys Lys	A2

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ACT CAA CAA GCT GCA CCT GTT GCA GAA ACA AAA ACT GAA GTA AAA CAA	528
Thr Gln Gln Ala Ala Pro Val Ala Glu Thr Lys Thr Glu Val Lys Gln	
165 170 175	
Thr Gln Gln Ala Ala Pro Val Ala Glu Thr Lys Thr Glu Val Lys Gln	(3); [39]
Glu Val Lys Gln	(2h); [33]
Gln Gln Ala Ala Pro Ala Ala Glu Thr Lys	52-10

ACT ACA CAA GCA ACT ACA CCT GCG CCT AAA GTA GCA GAA ACG AAA GAA	576
Thr Thr Gln Ala Thr Thr Pro Ala Pro Lys Val Ala Glu Thr Lys Glu	
180 185 190	
Thr Thr Gln Ala Thr Thr Pro Ala Pro Lys Val Ala Glu Thr Lys Glu	(3); [39]
Thr Thr Gln Ala Thr Thr Pro Ala	(2h); [33]
Thr Thr Gln Ala Thr Thr Pro Ala Pro Lys Val Ala	(2b); [27]
Thr Gln Ala Thr Thr Pro Ala	111b; [18]
X3 Thr Gln Ala Thr Thr Pro Ala X4	IVb
Pro Lys Val Ala Glu Thr Lys Glu	52-9
Glu Thr Lys Glu	52-8

ACT CCA GTA ATA GAT CAA AAT GCT ACT ACA CAC GCT GTC AAA AGC GGT	624
Thr Pro Val Ile Asp Gln Asn Ala Thr Thr His Ala Val Lys Ser Gly	
195 200 205	
Thr Pro Val Ile Asp Gln Asn Ala Thr Thr His Ala Val Lys Ser Gly	(3); [39]
Thr Pro	52-9
Thr Pro Val Val Asp Gln Asn	52-8

GAC ACT ATT TGG GCT TTA TCC GTA AAA TAC GGT GTT TCT GTT CAA GAC	672
Asp Thr Ile Trp Ala Leu Ser Val Lys Tyr Gly Val Ser Val Gln Asp	
210 215 220	
Asp Thr Ile Trp Ala Leu Ser Val Lys Tyr Gly Val Ser Val Gln Asp	(3); [39]

ATT ATG TCA TGG AAT AAT TTA TCT TCT TCT TCA ATT TAT GTA GGT CAA	720
Ile Met Ser Trp Asn Asn Leu Ser Ser Ser Ser Ile Tyr Val Gly Gln	
225 230 235 240	
Ile Met Ser Trp Asn Asn Leu Ser Ser Ser Ser Ile Tyr Val Gly Gln	(3); [39]

AAG CTT GCT ATT AAA CAA ACT GCT AAC ACA GCT ACT CCA AAA GCA GAA	768
CAA ACT GCT AAC ACA GCT ACT	11d; [12]
ACT GCT AAC ACA GCT	1d; [4]
X1 ACT GCT AAC ACA GCT X2	Vd
Lys Leu Ala Ile Lys Gln Thr Ala Asn Thr Ala Thr Pro Lys Ala Glu	
245 250 255	
Lys Leu Ala Ile Lys Gln Thr Ala Asn Thr Ala Thr Pro Lys Ala Glu	(3); [39]
Leu Ala Ile Lys Gln Thr Ala Asn Thr Ala Thr	(2c); [28]
Ala Ile Lys Gln Thr Ala Asn Thr Ala Thr Pro Lys	(2f); [34]
Ala Ile Lys Gln Thr Ala Asn Thr Ala	111c; [19]
X3 Ala Ile Lys Gln Thr Ala Asn Thr Ala X4	IVc
Lys Leu Ala Ile Lys Gln Thr Ala Asn Thr Ala Thr Pro Lys	Mono3-Pep

GTG AAA ACG GAA GCT CCA GCA GCT GAA AAA CAA GCA GCT CCA GTA GTT	816
Val Lys Thr Glu Ala Pro Ala Ala Glu Lys Gln Ala Ala Pro Val Val	

Listeria iap-/p60

260				265				270									
Val	Lys	Thr	Glu	Ala	Pro	Ala	Ala	Glu	Lys	Gln	Ala	Ala	Pro	Val	Val	(3);	[39]
AAA	GAA	AAT	ACT	AAC	ACA	AAT	ACT	GCT	ACT	ACA	GAG	AAA	AAA	GAA	ACA		864
Lys	Glu	Asn	Thr	Asn	Thr	Asn	Thr	Ala	Thr	Thr	Glu	Lys	Lys	Glu	Thr		
		275						280				285					
Lys	Glu	Asn	Thr	Asn	Thr	Asn	Thr	Ala	Thr	Thr	Glu	Lys	Lys	Glu	Thr	(3);	[39]
GCA	ACG	CAA	CAA	CAA	ACA	GCT	CCT	AAA	GCA	CCA	ACA	GAA	GCT	GCA	AAA		912
Ala	Thr	Gln	Gln	Gln	Thr	Ala	Pro	Lys	Ala	Pro	Thr	Glu	Ala	Ala	Lys		
		290					295				300						
Ala	Thr	Gln	Gln	Gln	Thr	Ala	Pro	Lys	Ala	Pro	Thr	Glu				(3);	[39]
		Gln	Gln	Gln	Thr	Ala	Pro	Lys	Ala	Pro	Thr	Glu				(2d);	[29]
		Gln	Gln	Thr	Ala	Pro	Lys	Ala	Pro	Thr						111d;	[20]
	X3	Gln	Gln	Thr	Ala	Pro	Lys	Ala	Pro	Thr	X4					IVd	
		Gln	Gln	Thr	Thr	Thr	Lys	Ala	Pro	Thr						111i;	[25]
	X3	Gln	Gln	Thr	Thr	Thr	Lys	Ala	Pro	Thr	X4					IVi	
Glu	Gln	Gln	Thr	Thr	Thr	Thr	Lys	Ala	Pro	Thr	Gln					(5d);	[38]
Gln	Gln	Gln	Thr	Ala	Pro	Lys	Ala	Pro	Thr	Glu						A486	
CCA	GCT	CCT	GCA	CCA	TCT	ACA	AAC	ACA	AAT	GCT	AAT	AAA	ACG	AAT	ACA		960
Pro	Ala	Pro	Ala	Pro	Ser	Thr	Asn	Thr	Asn	Ala	Asn	Lys	Thr	Asn	Thr		
305					310				315					320			
														Thr		56-9	
AAT	ACA	AAT	ACA	AAC	AAT	ACT	AAT	ACA	CCA	TCT	AAA	AAT	ACT	AAT	ACA		1008
Asn	Thr	Asn	Thr	Asn	Asn	Thr	Asn	Thr	Pro	Ser	Lys	Asn	Thr	Asn	Thr		
				325					330					335			
Asn	Thr	Asn	Thr	Asn	Asn	Thr	Asn	Thr	Asn							56-9	
AAC	TCA	AAT	ACT	AAT	ACG	AAT	ACA	AAC	TCA	AAT	ACG	AAT	GCT	AAT	CAA		1056
Asn	Ser	Asn	Thr	Asn	Thr	Asn	Thr	Asn	Ser	Asn	Thr	Asn	Ala	Asn	Gln		
			340					345				350					
GGT	TCT	TCC	AAC	AAT	AAC	AGC	AAT	TCA	AGT	GCA	AGT	GCT	ATT	ATT	GCT		1104
			C	AAT	AAC	AGC	AAT	TCA	AGT	GC						Ile;	[13]
			AT	AAC	AGC	AAT	TCA	AG								Ie;	[5]
		X1	AT	AAC	AGC	AAT	TCA	AG	X2							Ve	
Gly	Ser	Ser	Asn	Asn	Asn	Ser	Asn	Ser	Ser	Ala	Ser	Ala	Ile	Ile	Ala		
		355					360					365					
GAA	GCT	CAA	AAA	CAC	CTT	GGA	AAA	GCT	TAT	TCA	TGG	GGT	GGT	AAC	GGA		1152
Glu	Ala	Gln	Lys	His	Leu	Gly	Lys	Ala	Tyr	Ser	Trp	Gly	Gly	Asn	Gly		
	370					375					380						
CCA	ACT	ACA	TTT	GAT	TGC	TCT	GGT	TAC	ACT	AAA	TAT	GTA	TTT	GCT	AAA		1200
Pro	Thr	Thr	Phe	Asp	Cys	Ser	Gly	Tyr	Thr	Lys	Tyr	Val	Phe	Ala	Lys		
385					390				395						400		

Listeria iap-/p60

GCG GGT ATC TCC CTT CCA CGT ACA TCT GGC GCA CAA TAT GCT AGC ACT 1248
 Ala Gly Ile Ser Leu Pro Arg Thr Ser Gly Ala Gln Tyr Ala Ser Thr
 405 410 415

ACA AGA ATT TCT GAA TCT CAA GCA AAA CCT GGT GAT TTA GTA TTC TTC 1296
 Thr Arg Ile Ser Glu Ser Gln Ala Lys Pro Gly Asp Leu Val Phe Phe
 420 425 430

GAC TAT GGT AGC GGA ATT TCT CAC GTT GGT ATT TAT GTT GGT AAT GGT 1344
 Asp Tyr Gly Ser Gly Ile Ser His Val Gly Ile Tyr Val Gly Asn Gly
 435 440 445

CAA ATG ATT AAC GCG CAA GAC AAT GGC GTT AAA TAC GAT AAC ATC CAC 1392
 Gln Met Ile Asn Ala Gln Asp Asn Gly Val Lys Tyr Asp Asn Ile His
 450 455 460

GGC TCT GGC TGG GGT AAA TAT CTA GTT GGC TTC GGT CGC GTA TAA 1437
 TT GGC TTC GGT CGC GTA GAA TTC ATA
 GC TTC GGT CGC GTA
 X1 GC TTC GGT CGC GTA X2
 Gly Ser Gly Trp Gly Lys Tyr Leu Val Gly Phe Gly Arg Val +
 465 470 475
 I1b; [10]
 Ib; [2]
 Vb